during evolution and it causes variety of genetic changes in the modern human genome. The role of human ORF1p (hORF1p) in LINE1 retrotransposition is largely unknown, although it presumably involves its nucleic acid chaperone activity and its ability to oligomerize on nucleic acids. To better understand the molecular mechanism of hORF1p in LINE1-retrotransposition, we developed a novel method to characterize single-stranded DNA (ssDNA)-hORF1p interactions using single molecule stretching with optical tweezers. Here we examined the interactions of two hORF1p variants with ssDNA: hORF1-111p, the modern wild type (wt) protein and hORF1-151p, a hybrid of modern wt and a resuscitated ancestral hORF1p. Although the *in vitro* nucleic acid chaperone activities are indistinguishable in the two variants, 151p is inactive in an *in vivo* retrotransposition assay. We characterized three distinct binding kinetics for 111p and 151p with ssDNA. A fast kinetic fraction characterized by association and dissociation on a timescale of seconds, an intermediate fraction with a timescale of greater than one minute, which characterizes dissociation of the protein after the stretching force on DNA is released and a slow fraction with negligible dissociation on a timescale of tens of minutes. The fast fractions of both variants are converted to intermediate and slow fractions with time, consistent with protein oligomerization. However, oligomerization of 151p occurs two orders of magnitude slower than 111p. This result could explain the inactivity of 151p in retrotransposition and suggests that the oligomerization rate of ORF1p is important for retrotransposition.

1026-Plat

Nucleosome Assembly Dynamics Involve Spontaneous Fluctuations in the Handedness of Tetrasomes

Rifka Vlijm[1], Mina Lee[1], Orkide Ordu[1], Jan Lipfert[1], Alexandra Lusser[2], Nynke Dekker[1], Cees Dekker[1].
[1]Departmnent of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, Netherlands, [2]Division of Molecular Biology, Innsbruck Medical University, Biocenter, Innsbruck, Netherlands.

Nucleosomes play crucial roles in eukaryotic cells as they form the basic compaction unit of DNA, and impact the accessibility of the DNA for transcription and replication. How the assembly, stability, and disassembly of nucleosomes affect the interplay between compaction and accessibility forms a question of key interest. Here we use freely-orbiting magnetic tweezers (FOMT), which can measure in real time the changes in length and twist of individually tethered DNA molecules. We monitor how Nucleosome Assembly Protein-1 (NAP1) loads either histone tetramers (an important intermediate in dis/assembly or remodeling) or complete histone octamers onto DNA, thus forming nucleosomes. Remarkably, tetrasomes are observed to exhibit a spontaneous flipping between DNA states with linking number $\Delta Lk=-0.73$ and $\Delta Lk=+1.0$, without concomitant changes in DNA end-to-end length. Such behavior is absent in the case of nucleosomes. The statistics of tetrasome flipping shows that the preferentially occupied left-handed state has a probability $p=0.9$. The difference in free energy between these two states is thus 2.3kBT. We also demonstrate how controlled application of positive torque can drive tetrasome occupancy from left-handed to right-handed states. To exclude effects of the assembly protein NAP1, we repeated these experiments with tetrasomes assembled in the conventional way using salt dialysis. These results show that the spontaneous fluctuations in the handedness of tetrasomes are not NAP1 induced. Since we expect the H3-H3 interface to play an important role in the handedness fluctuations, we also studied tetrasomes in which the replication-coupled canonical histone H3 was replaced by the replication-independent variant H3.3, which show very similar behavior. Our findings thus reveal unexpected dynamical rearrangements of the nucleosomal structure that suggest that chromatin can serve as a 'twist reservoir', offering a mechanistic explanation for the regulation of DNA supercoiling in chromatin.

1027-Plat

Facilitated Dissociation of Protein from a Single DNA Binding Site

Ramsey I. Kamar[1], John F. Marko[1,2].
[1]Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA, [2]Physics and Astronomy, Northwestern University, Evanston, IL, USA.

Protein-DNA interactions are central to chromosome compaction, defining the topology of chromatin, DNA repair, and gene regulation, and therefore control all aspects of cellular function. It is therefore important to understand the factors that regulate the dynamics of protein-DNA interactions since these factors will influence the dynamics of cellular processes. Previous single DNA studies involving the major nucleoid-associated protein Fis have shown that it stably binds 48.5 kb DNA in Fis-free buffer. However, protein in solution was found to accelerate the off-rate of Fis in a concentration dependent manner via an unknown mechanism. This effect has also recently been observed in a number of other cases suggesting its generality. It is unknown whether facilitated dissociation is an effect at the single binding site level or whether it involves protein clustering and/or cooperativity. Using single molecule fluorescence microscopy, we have measured the off-rate of Fis from 27bp dsDNAs, constituting individual Fis binding sites, and have observed facilitated exchange demonstrating an effect at the single binding site level. We have also found that the salt dependence of the off-rate is dramatically reduced when protein is in solution. This observation provides strong support for a simple microscopic theory that thermally excited, partial dissociation events lead to facilitated dissociation. In the absence of cooperativity, it is commonly assumed that finding the ratio of the off-rate to the on-rate constant will produce the same dissociation constant as finding the concentration of ligand for which half of the substrates are bound by ligand. However, if the off-rate is a function of protein concentration, this equivalence no longer holds. If facilitated exchange is found to be a general phenomenon, it will force a change in how we think about protein binding kinetics in biology.

1028-Plat

Protein-DNA Binding in the Absence of Consensus Binding Motif

Ariel Afek[1], Joshua L. Schipper[2], Raluca Gordân[2], David B. Lukatsky[1].
[1]Department of Chemistry, Ben-Gurion University, Beer sheva, Israel, [2]Department of Biostatistics and Bioinformatics, Duke University, Durham, NC, USA.

Until now, it has been reasonably assumed that specific base-pair recognition is the only mechanism controlling the specificity of transcription factor (TF)-DNA binding. In our study we show that nonspecific DNA sequences possessing certain repeat symmetries, when present outside of specific TF binding sites (TFBSs), statistically control TF-DNA binding preferences. We used high-throughput protein-DNA binding assays to measure the binding levels for several human TFs to tens of thousands of short DNA sequences with varying repeat symmetries. Based on statistical mechanics modeling, we identify a new protein-DNA binding mechanism induced by DNA sequence symmetry in the absence of consensus binding motif, and experimentally demonstrate that this mechanism indeed highly affects protein-DNA binding preferences.

1029-Plat

Mapping Lac Repressor Interactions along DNA with Ultra-Fast Optical Tweezers

Alessia Tempestini[1,2], Carina Monico[3], Francesco Vanzi[1], Francesco Saverio Pavone[1], Marco Capitanio[1].
[1]LENS, Università degli studi di Firenze, Sesto Fiorentino, Italy, [2]LENS, Sesto Fiorentino, Italy, [3]Università of Oxford, Oxford, United Kingdom.

Transcription factors and DNA-binding proteins bind their specific target sequences with rates higher than allowed by 3D diffusion alone. Generally accepted models predict a combination of free 3D diffusion and 1D sliding along non-specific DNA [1]. One important issue in the field of protein-DNA interaction is the understanding of how proteins interact with non-cognate DNA sequences and how they find the sequence of interest along the DNA. We developed a system that permits to detect protein-DNA interaction with sub-ms temporal resolution and nanometer spatial precision [2]. Such precision allows us to discriminate between short events (milliseconds), which are non-specific, and long events (tens of seconds) considered to be highly specific, and to determine the precise location along the DNA where they occur. We chose a well-known example of gene expression regulation, based on the specific interaction of Lac repressor protein (LacI) with its target DNA sequence (operator). We used a DNA molecule containing two copies of the O1 and one of the O3 operator placed at known distances and we obtained a map of the long interactions along the DNA molecule, corresponding to the position of the two O1 operators. Short interactions were instead spread along the whole DNA molecule but occurred with higher probability in correspondence of the operators and in their proximity. Dissociation of both classes of interactions was highly accelerated by an external load. Measurements performed in the presence of IPTG, a mimic of the inducer allactose, resulted in increased dissociation from the lac operators.

[1] Monico, C. et al., Int. J. Mol. Sci. 14, 3961-3992 (2013).
[2] Capitanio, M. et al., Nature Methods, 9, 1013-1019 (2012)

1030-Plat

Direct Observation of TALE Protein Search Dynamics along DNA

Luke W. Cuculis[1], Zhanar Abil[2], Huimin Zhao[3], Charles M. Schroeder[3].
[1]Department of Chemistry, University of Illinois Urbana-Champaign, Urbana, IL, USA, [2]Department of Biochemistry, University of Illinois Urbana-Champaign, Urbana, IL, USA, [3]Department of Chemical and Biomolecular Engineering, University of Illinois Urbana-Champaign, Urbana, IL, USA.

In this work, we discuss the direct observation of transcription activator-like effector (TALE) protein dynamics along DNA templates using single molecule